

### **ABSTRACT OF THE DISCLOSURE**

A method of calibration of magnification of a microscope with the use of a diffraction grating has the steps of determining a mean period of a diffraction grating by irradiating the diffraction grating with an electromagnetic radiation having a known wavelength and analyzing a resulting diffraction pattern, determining a scatter of individual values of a period of the diffraction grating by multiple measurements of periods of the diffraction grating by a microscope in pixels in one area in a microscope field of view, and calculating a mean value of the period and the scatter based on the measurements, determining a sufficient number of measurements of the period for providing an accepted statistic error of a magnification of the microscope, performing measurements corresponding to the determined acceptable number of measurements, of individual values of the period in pixels in a plurality of portions of the diffraction grating, calculating a general mean value of the period in pixels based on the immediately preceding step, and finally calculating a parameter corresponding to the magnification of the microscope based on the determined mean value of the period of the diffraction grating in the microscope image and the calculating of the general mean value of the period in pixels.